

DETECTION OF TUMOR RNA IN PLASMA AND SERUM

This application is a continuation-in-part of U.S. Patent Application, Serial No. 09/155,152, filed September 22, 1998, the entire disclosure of which is hereby incorporated by reference.

BACKGROUND OF THE INVENTION

1. Field of the Invention

This invention relates to methods for detecting tumor-derived or tumor-associated mammalian ribonucleic acid (RNA) in bodily fluids such as blood plasma and serum obtained from an animal, most preferably a human. Specifically, the invention is directed towards methods for detecting RNA in bodily fluids from a human bearing a premalignant lesion or a malignancy, ranging in severity from localized neoplasia to metastatic disease. The methods of the invention are particularly drawn to detecting RNA encoding all or a portion of particular genes associated with neoplastic growth, development, or pathogenesis. In particular, these methods are drawn to genes associated with tumor growth factors such as tyrosine kinase mediated growth factors (*for example*, epidermal growth factor, EGF) and their receptors (*for example*, epidermal growth factor receptor (EGFr), and her-2/neu), as well as oncogenes such as c-myc oncogene. The methods of the invention are further particularly drawn to detecting RNA derived or associated with tumor-associated ribonucleoprotein, such as but not limited to heterogeneous nuclear ribonucleoprotein A2/B1 (hnRNP A2/B1) and associated ribonucleoprotein. In view of the essential role of RNA in expressing genes and producing proteins encoded thereby, detection and monitoring of RNA provides a convenient and reliable method for assessing and monitoring gene expression associated with neoplastic disease, thereby enabling the detection, diagnosis, monitoring, evaluation, and prognosticating of cancer and premalignancy.

2. Background of the Related Art

The pathogenesis and regulation of cancer is dependent upon gene expression, comprising production and translation of RNA to produce proteins involved with a variety of cellular processes, such as cell proliferation, regulation, and death. Furthermore, some gene expression, resulting in the existence of RNA and the proteins translated therefrom in cells and tissues, although not necessarily involved in neoplastic pathogenesis or regulation, may comprise a phenotype of recognizable characteristics for particular neoplasms, *for example*, either by being expressed at elevated levels or by being inappropriately expressed in said cells or tissues.

Tyrosine kinase-mediated growth factors and their receptors such as epidermal growth factor (EGF), epidermal growth factor receptor (EGFr), and her-2/*neu*, play important roles in the growth of many epithelial cancers and their response to insult or injury. Oncogenes such as c-myc play important roles in the pathogenesis of many cancers. Other proteins, such as hnRNP A2/B1 and associated ribonucleoproteins including hnRNP A2 (heterogeneous nuclear ribonucleoprotein A2) and hnRNP B1 (heterogeneous nuclear ribonucleoprotein B1) are overexpressed early in the development of some cancers. Detection of RNA encoding EGF, EGFr her-2/*neu*, c-myc or hnRNP A2/B1 provides a method for detecting and monitoring a wide spectrum of cancers and premalignancies, and can have prognostic significance. Tyrosine kinase-mediated growth factors and their receptors further provide potential targets for cancer therapies such as monoclonal antibody-based therapies (*for example*, herceptin for her-2/*neu* and CA-225 for EGFr), small molecule therapies and tyrosine kinase inhibitors, as well as vaccine therapies. Detection of EGF, EGFr and her-2/*neu* RNA can thus provide methods for selecting and monitoring patients for such therapies.

RNA associated with cancer and premalignant or neoplastic states, such as RNA encoding EGF, EGFr her-2/*neu*, c-myc or hnRNP A2/B1 are referred to herein as tumor-derived or tumor-associated RNA. Co-owned and co-pending U.S. Patent Application Serial No. 09/155,152, incorporated by reference herein in its entirety, provides methods by which mammalian tumor-associated or tumor-derived RNA in bodily fluids such as plasma and serum can be detected and utilized for detecting, monitoring, or evaluating cancer or premalignant conditions. U.S. Patent Application Serial No. 09/155,152, incorporated by reference herein in its entirety, further taught that tumor-associated or tumor-derived RNAs include *erb*-B-1 mRNA (also known as epidermal growth factor receptor mRNA), her-2/*neu* mRNA (also known as *erb*-B-2 mRNA), c-myc mRNA, and hnRNP A2/B1 associated RNA were advantageously detected in bodily fluids such as blood plasma or serum.

RNA encoding EGF, EGFr, her-2/*neu*, c-myc, and hnRNP A2/B1 being recognized as tumor-associated RNAs, there is a newly-appreciated need in the art to identify premalignant or malignant states characterized by said RNA in animals including humans by detecting said RNA in bodily fluids such as blood plasma or serum.

SUMMARY OF THE INVENTION

The present invention provides methods for detecting EGF RNA, EGFr RNA, her-2/*neu* RNA, and hnRNP A2/B1 RNA, or any combination thereof, in bodily fluids, preferably in blood and most preferably in blood plasma and serum, and in other bodily fluids including but not limited to urine, effusions, ascites, saliva, cerebrospinal fluid, cervical secretions, vaginal secretions, endometrial secretions, gastrointestinal secretions, sputum and bronchial secretions, and breast fluid and associated lavages and washings. The inventive methods comprise detecting